Remarks/Arguments

Reconsideration of the above-identified application in view of the present amendment is respectfully requested.

By the present amendment, claims 1, 24, 25, 26, 29, 54, and 57 have been amended and claims 64-66 have been cancelled. Support for amended claims 1, 54, and 57 can be found in Examples 3, 4, 6, and 11 as well as p17, lines 9-17 and 22-29; p20, lines 7-24; p29, lines 18-25.

Below is a discussion the 35 U.S.C. §103(a) rejection of claims 1-2, 4, 10-12, 21, 23-36, 40-43, 50-54, 56-57 and 62-69, and the patentability of new claims 70 and 71.

1. 35 U.S.C. §103(a) rejection of claims 1, 2, 4, 10-12, 21, 23-36, 40-43, 50-54, 56, 57, and 62-69.

Claims 1, 2, 4, 10-12, 21, 23-36, 40-43, 50-54, 56, 57, and 62-69 are rejected under 35 U.S.C. §103(a) as being unpatentable over Strauer *et al.* (2002, *Circulation* 106: 1913-1918) in view of Shake *et al.* (2002, *Annals of Thoracic Surgery* 73: 1919-1926), Ueno *et al.* (U.S. Patent Application Publication No. 2002/0037278), Kocher *et al.* (2001, *Nature Medicine* 7: 430-436), and Itescu (U.S. Patent Application Publication No. 2003/0199464).

Applicants respectfully submit that claims 1, 54, and 57 are patentable over Strauer *et al.* in view of Kocher *et al.*, Shake *et al.*, Ueno *et al.* and Itescu *et al.* because: (1) Strauer *et al.* in view of Kocher *et al.*, Shake *et al.*, Ueno *et al.* and Itescu *et al.* do not teach or suggest to one of ordinary skill in the art administering to a subject a therapeutically effective amount of a first enriched population of cells

comprising at least 75% human CD133+/CD34+ endothelial progenitor cells and a second enriched population of cells consisting essentially of human mesenchymal stem cells, and (2) Strauer *et al*. teach away from administering to a subject a therapeutically effective amount of a first enriched population of cells comprising at least 75% human CD133+/CD34+ endothelial progenitor cells and a second enriched population of cells consisting essentially of human mesenchymal stem cells.

Strauer *et al*. as discussed in the Office Action teach isolating bone marrow (BM) from human, isolating bone marrow mononuclear cells (BMCs) there from, cultivating them overnight, and administering over 10^6 BM-MNCs to the ischemic tissue using a balloon catheter. Strauer et al. also teach that 0.65% of the isolated BM-MNCs are AC133+ and that the BMCs include mesenchymal stem cells. Strauer et al., however, do not teach or suggest administering to a subject a therapeutically effective amount of a first enriched population of cells comprising at least 75% human CD133+/CD34+ endothelial progenitor cells and a second enriched population of cells consisting essentially of human mesenchymal stem cells. BMCs comprising 0.65% endothelial progenitor cells are not a first enriched population of cells comprising 75% human CD133+/CD34+ endothelial progenitor cells and/or a second enriched population of cells consisting essentially of human mesenchymal stem cells.

Shake et al. teach isolating MSCs from bone marrow and culturing them such that hematopoietic cells, fibroblasts, and non-MSC adherent cells are washed away, yielding a purified MSC culture. Shake et al., however, do not teach that an enriched

population of cells consisting essentially of MSCs can be administered in combination with another enriched population of cells let alone an enriched population of cells comprising 75% human CD133+/CD34+ endothelial progenitor cells.

Ueno et al. teach methods of treating ischemic tissues by administering bone marrow mononuclear cells. Ueno et al., however, do not teach or suggest administering to a subject a therapeutically effective amount of a first enriched population of cells comprising at least 75% human CD133+/CD34+ endothelial progenitor cells and a second enriched population of cells consisting essentially of human mesenchymal stem cells. A population of BMCs, which as noted in Strauer et al., comprising 0.65% endothelial progenitor cells, is not a first enriched population of cells comprising 75% human CD133+/CD34+ endothelial progenitor cells and/or a second enriched population of cells consisting essentially of human mesenchymal stem cells.

Kocher et al. teach that bone marrow derived angioblasts, which express AC133 and CD34, among other markers promote revascularization of infarcted myocardium. As discussed in the Office Action, Kocher et al. teach at page 435, column 1, paragraph 4 that CD34+ mononuclear cells isolated to 98% purity can express AC133. Specifically, Kocher et al. note on pages 430-431 that 60%-80% of CD34+ cells isolated to 98% purity can express CD117+. Of these CD34+/CD117+ cells, CD117 expression was bright in 15-25% of CD34+/CD117+ cells and dim in 75%-85% of CD34+/CD117+ cells. VEGFR-2 expression was detected at high density on 20-30% $CD117^{dim}$ and at lower density in 10-15% of $CD117^{bright}$ cells.

VEGFR-2+ cell were shown to also express AC133. Accordingly, Kocher et al. teach that of the mononuclear cells selected for CD34+ cells, less than 24% of the cells express AC133. (% of CD34+ cells that are CD117+ x % of $CD117^{dim}$ and $CD117^{bright}$ cells that are VEGFR-2+). Kocher et al. further note that populations of either CD34+ cells, CD34/CD117+ cells, or CD34+/$CD117^{bright}$ cells were administered to a subject to promote angiogenesis. None of these populations, however, included at least 75% human CD133+/CD34+ endothelial progenitor cells. Moreover, Kocher et al. do not teach administering a second enriched population of cells consisting essentially of human mesenchymal stem cells in combination with a first enriched population of cells comprising at least 75% human CD133+/CD34+ endothelial progenitor cells.

Itsecu, as discussed in the Office Action, teaches methods of regeneration myocardial tissue after ischemic tissue by administering endothelial progenitor cells that can express CD117, CD34, AC133, or a high level of intracellular GATA activity. Itsecu teach in the Examples from paragraphs 106+ that a population of CD34+ cells of 98% purity comprising 6-12% $CD117^{bright}$ cells can be administered to a subject. Itsecu, however, does not teach or suggest administering to a subject a therapeutically effective amount of a first enriched population of cells comprising at least 75% human CD133+/CD34+ endothelial progenitor cells and a second enriched population of cells consisting essentially of human mesenchymal stem cells. Itsecu teaches only that the mononuclear cells can be selected for CD34+ and that these selected and purified cells include at least some cells that include CD133+ markers. Itsecu does not teach selecting for CD133+ and CD34+ to achieve an

enriched population of cells that include at least 75% human CD133+/CD34+ endothelial progenitor cells let alone using this population in a therapeutic application in combination with a second enriched population of cells consisting essentially of mesenchymal stem cells.

Accordingly, none of the references cited in the Office Action teach or suggest administering to a subject a therapeutically effective amount of a first enriched population of cells comprising at least 75% human CD133+/CD34+ endothelial progenitor cells let alone a first enriched population of cells comprising at least 75% human CD133+/CD34+ endothelial progenitor cells in combination with a second enriched population of cells consisting essentially of human mesenchymal stem cells.

The Office Action suggests that one skilled in the art would have a reasonable expectation of success in enriching CD34+CD133+ EPCs with the BMC-NCs of Strauer et al. at least two-fold because Kocher et al. teach methods of enriching such cells to 98% purity and Kocher et al. recognized that CD133+ cells promote neovascularization of ischemic tissue. In contrast to the Office Actions assertions and as discussed above, Kocher et al. only teach enriching CD34+ and CD34+CD117^{bright} cells and do not provide any evidence or teaching that it is desirable to enrich CD34+CD133+ cells to provide an enriched population comprising at least 75% CD34+CD133+ cells. Moreover, Applicants fail to see where in Kocher it is stated that CD133+ cells can promote neovascularization. Kocher teaches a mixed population of CD34+ cells (page 432) and/or a population of

CD34+/CD117^{bright}/GATA-2^{HI} can promote neovasuclarization but these populations at best include less than 24% CD34+/CD133+ cells.

Moreover, Strauer et al. teach away from administering an enriched CD34+CD133+ cell population to a subject. Strauer et al. teach administering a heterogeneous BM-MNC population. Strauer et al. reference Kocher et al. to indicate that other groups have found that component cell types of the BM-MNC population may differentiate into cell types that might be beneficial to an infarct heart, but Strauer *et al*. reason that "several different fractions of mononuclear BMCs may contribute to the regeneration of necrotic myocardium and vessels" and "in order to utilize this large and perhaps heterogeneous regenerative potential, [Strauer *et al*.] decided to use all mononuclear cells from the bone marrow aspirate as a whole, rather than a subpopulation" (p1917, col. 1, para. 1). Additionally, Strauer *et al*. also teach away from enriching a population of EPCs by teaching that *in vitro* propagation of BM-MNCs leads to attenuated homing ability. The Office Action states that Strauer *et al*. does not teach enriching CD34+/CD133+ EPCs at least two-fold prior to administration to the subject. In fact, Strauer *et al*. state that that no further expansion (i.e. enrichment) was performed because "prior experimental data have revealed a dramatic decline in the homing capacity of *in vitro* amplified hematopoietic stem or progenitor cells" (p1917 , Col. 1, Para. 1, emphasis added). Accordingly, Strauer et al. teach using a heterogeneous population of cells and teach away from administering to a subject a first enriched population of cells comprising at least 75% CD34+/CD133+ EPCs.

Accordingly, Applicants respectfully submit that claims 1, 54, and 57 are patentable over Strauer *et al.* in view of Kocher *et al.*, Shake *et al.* and Ueno *et al.*, and request that the 35 U.S.C. §103(a) rejection of the claim be withdrawn. Additionally, Applicants respectfully request that the 35 U.S.C. §103(a) rejection of claims that depend either directly or indirectly form claims 1, 54, and 57 be withdrawn.

Claim 2, 4, 10-12, 21, 23-36, 40-43, 50-53, 56, and 67-69 depend respectively from claims 1, 54, and 57 and are therefore allowable because of the aforementioned deficiencies in the rejections with respect to claims 1, 54, and 57 and because of the specific limitations recited in claims 1, 54, and 57.

In view of the foregoing, it is respectfully submitted that the present application is in a condition of allowance and allowance of the present application is respectfully requested.

Please charge any deficiency or credit any overpayment in the fees for this matter to our Deposit Account No. 20-0090.

Respectfully submitted,

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